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TRITIUM FRACTIONATION IN PLANTS

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National Environmental Research Center
Las Vegas, Nevada

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ABSTRACT

Alfalfa plants were hydroponically grown in environmental growth chambers in which they were continuously exposed to tritium throughout growth. All segments of the environment were in equilibrium with respect to the specific activity of tritium. The tritium content in plant organic matter was about 22 percent lower than in the plant free water or rooting solution. Under conditions of low transpiration, there was a higher concentration (about 1.8 percent) of tritium in the leaves than in the stems and rooting solution. This is thought to represent the result of fractionation during transpiration.

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INTRODUCTION

In 1931, Urey, Brickwedde, and Murphy (16) demonstrated the existence of deuterium by separating it from ordinary hydrogen and analyzing it in a mass spectrometer. This discovery was due to different masses which resulted in the separation or fractionation of the two isotopes. The importance of this mass difference, and the possibility of fractionation of hydrogen isotopes in biological systems has been of concern since that time. The thermodynamic basis for isotopic fractionation was presented by Urey and Rittenberg (15) only two years after its discovery. Additional considerations of isotope fractionation have been detailed by several authors including Bigeleisen (3, 4), Wiberg (20), Salomon (12), and Weston (19). Because water is the most abundant hydrogen containing molecule and is essential to all living organisms, it has been the object of most fractionation studies. In plants, water functions as a reactant, temperature regulator, and transport medium. Fractionation may be expected at several sites, not particularly dependent on its position or function, but rather on the type of reaction, degree of compartmentation, and on the source of energy causing the reaction. In general, the mass flow of water is unaffected by isotope differences since the energy for transport is remote from the molecules being moved. This does not eliminate the possibility of observing fractionation along a water transport continuum in soil or plants. But if observed, it is clear that fractionation must have resulted from reactions such as exchange or dilution. When reactions depend on the molecular-free energy of the hydrogen-containing species, fractionation is always possible. This occurs in chemical reactions and other processes such as exchange and evaporation which are the result of molecular diffusion.

When fractionation occurs between molecules containing tritium or deuterium, and those with normal hydrogen, there are always two results: (1) the newly formed product is depleted in the heavier isotope, and (2) the substrate is enriched. If the substrate pool is large or is rapidly being replenished, the effect of isotopic fractionation can be observed by separating and analyzing both segments. But if the substrate is formed at the same rate as the product, such as in a chain of reactions, the concentration of the heavy isotope in the substrate increases until a steady state is reached and an equilibrium concentration is achieved. The existence of this steady state fractionation is difficult to observe because the size of the substrate pool is often so small that it precludes isolation, and because the net or overall fractionation is very small.

It was shown by Stewart (14) that a characteristic fractionation occurs in the exchange of hydroxide ions from water to specific clays. None was observed in water percolation. Although this fractionation may be important in identifying the age of soil water where the flow rate is slow, it is generally concluded (Zimmermann, 22) that, in an agricultural setting, fractionation in soil water has an insignificant impact in terms of tritium availability to plants.

Reports of hydrogen isotope fractionation in plants started in 1934 (Washburn and Smith, 17) only three years after the first isolation of deuterium. Since then, many reports have been published giving evidence of hydrogen isotopic fractionation in plants. Reports of Washburn and Smith (17) and Zimmerman et al. (21) indicate that no fractionation occurs during transpiration; however, Wershaw et al. (18) reported finding that deuterium was greatly enriched in the leaves of trees with respect to the water in the xylem and phloem. Most studies have indicated the existence of an isotopic fractionation against the heavy hydrogen isotopes being incorporated into the organic molecules of plants. Smith and Epstein (13) showed that in marsh plants a 4.4 percent

fractionation occurred in the formation of organic compounds. In addition, fractionation of 9.2 percent occurred between carbohydrates and lipids. An apparent exception was demonstrated by Helvey (6) when he showed that honey had a higher deuterium content than the apparent water source. Aronoff and Choi (1) showed that sugar synthesis in green plants used a substrate which was in rapid equilibrium with water, but that hydrogen from some other sources not in equilibrium with water was also used. This resulted in the water having a higher tritium content than the sugars in short term experiments.

A literature review by Bruner (5) contains lists of data from studies comparing the tritium content in various segments of plants and animals. Some data support the idea of discrimination against heavy hydrogen, some suggest preferential accumulation, and some report no fractionation. Thus, as a result of the conflicting general summaries reported, there is considerable confusion in the literature regarding hydrogen isotope fractionation in biological systems.

Difficulties in sample preparation and experimental design have made a complete understanding of fractionation difficult. Problems which are most often overlooked in plant research, and which are most often the source of serious errors, are caused by failing to understand the rapid exchange which can occur between tritium in water, either in solution or vapor, and the OH groups, especially on carbohydrates (Lang and Mason, 8). This may partially account for the lack of agreement in the literature regarding the extent and nature of hydrogen isotope fractionation. Field studies are especially plagued by another problem: the interaction of time on the interpretation of results. It is tempting to analyze a plant or animal for tritium existing in various compartments and assign differences to isotopic fractionation. However, it is obvious, but sometimes forgotten, that compartmentation is based on both a physical and time separation.

The purpose of this work was to carefully investigate the fractionation of tritium from protium in the formation of organic molecules in plants grown in a controlled, uniformly tritiated environment. Also, the experiment was designed to measure any fractionation which may occur during transpiration and absorption.

METHODS

Alfalfa plants (Medicago sativa) were grown in an environmental simulation chamber from seedlings to mature plants. The chamber was designed to eliminate air leakage (Hill, 7) and thus allowed the maintenance of a uniformly tritiated environment throughout growth. Radiant energy was produced by cool white fluorescent and incandescent lamps and yielded a quantrum flux of 325 $\mu E/m^2$ in the wave band from 400-700 nm at the level of the plant containers. The temperature was maintained at 25 ± 0.5°C during the day and 20 ± 0.5°C at night. Relative humidity was 70 ± 4 percent. Carbon dioxide was monitored continuously and maintained at 350 ± 15 PPM by automatic metered injections of CO_2. Humidity was controlled by the temperature of a cold radiator and the condensate was collected and reused to fill the hydroponic containers. Thus, the water moved in a cycle through the plants into the air, and returned to the rooting medium. The tritium concentration of the water was monitored periodically, and when additional water was added to replace water lost in harvesting, it was made up at the same concentration of tritium. Once every two months, the buckets containing the hydroponic solution were drained and cleaned to remove organic debris and refilled with fresh hydroponic solution (Berry, 2). In the interim, the nutrient content of the solution was maintained by measuring pH and electrical conductivity and adding water or nutrients as needed.

The alfalfa plants used in these studies were grown in support of another project (Moghissi et al. 11) and were only

sampled for fractionation evaluation after reaching maturity from the previous harvest in an undisturbed chamber (about five weeks).

When harvested, plants were immediately placed into benzene. Water was extracted by heating the benzene to form a water/benzene azeotrope, and was collected by cooling the distillate (Moghissi et al., 11). This collection system has been shown to be free of isotopic discrimination which is an extremely important consideration not true with many drying systems (McFarlane et al., 9). The plants were dried of benzene in desiccators under vacuum with great care being exercised to eliminate contact with the ambient environment. This precaution was taken to eliminate the possibility of hydrogen exchange from water vapor and cellulose OH groups. When dry, the plants were transferred to a stainless steel container (Parr bomb) ignited in an O_2 atmosphere of 500 psi. The water of combustion was collected by bleeding the air from the bomb slowly through a liquid nitrogen trap. Water samples were analyzed by liquid scintillation counting (Moghissi, 10).

RESULTS AND DISCUSSION

The concentrations of tritium in plant-free water, gross organic structure, and in the nutrient solution are shown in Table I. These results have been normalized to reflect the concentration of the hydroponic solution as being 100 percent. The experiment was repeated three times with four replicates at each harvest. Although the hydroponic solutions were slightly different between harvest dates, the coefficient of variation of the replicated hydroponic solution was less than 0.7 percent in all cases. Records of the tritium concentration in the hydroponic solution were kept throughout the growth. The concentration did not vary more than ± 1 percent from the time of harvesting the last crop until harvesting the experimental samples. Using all 12 sets of data, paired T tests showed significant differences

Table I. RELATIVE TRITIUM CONCENTRATIONS IN PLANTS GROWN IN A CONSTANT AND UNIFORMLY CONTAMINATED ENVIRONMENT+

Hydroponic Solution	Plant Free H_2O	Water from Oxidation of Organic Hydrogen
nCi/ml	nCi/ml	nCi/ml
100.0 ± 0.2*	97.4 ± 0.6	77.5 ± 1.9

* Based on paired T tests of 12 replicates, the mean values are significantly different from each other at the 0.001 level.

± The 95% confidence interval.

\+ Normalized to hydroponic solution.

between the concentration of tritium in the free water and organic portion at the .001 level.

The fractionation between the free water and the organic constituents of the plant is extremely difficult to evaluate in the field and in most experimental conditions because the plant water is rapidly exchanged with the water source. Exchange of water in the atmosphere with plants also compounds the interpretation of fractionation data. In these experiments where the environment was carefully controlled to eliminate these variables, the tritium fractionation between free water and organics was about 22 percent.

Fractionation in evaporation is familiar to all who have tried to collect water from plants and soil by any of the evaporation methods. It may therefore be surprising to find that no fractionation has been consistently reported in plant transpiration. Transpiration is a diffusive phenomenon dependent upon molecular free energy of the water molecule. Thus, it falls into the category of reactions subject to fractionation. The supply of water to the evaporative surface is by mass flow and is not expected to be subject to fractionation. In this situation, fractionation would only be evident if diffusion of heavy water away from the evaporative site was sufficiently rapid to increase the concentration of tritium in the surrounding tissue. When transpiration is rapid, the mass flow of water toward the evaporation site is so fast that it counteracts the diffusive movement of tritiated water away from the site. Thus, under conditions of rapid transpiration, fractionation would not be evident. The data in Table II show that when all conditions remained constant except the rate of transpiration, which was decreased by increasing the humidity, there was an apparent fractionation between the water in the stem and leaves of alfalfa plants. This was thought to be evident because of a decrease in the ratio of evaporation to diffusion away from the site.

Table II. TRITIUM CONCENTRATION IN FREE WATER OF ALFALFA STEMS AND LEAVES UNDER CONDITIONS OF SLOW AND RAPID TRANSPIRATION

	SLOW TRANSPIRATION (High Relative Humidity > 70%) nCi/ml	RAPID TRANSPIRATION (Low Relative Humidity < 25%) nCi/ml
Leaves	77.2 ± 0.6†	75.4 ± 0.5
Stems	75.4 ± 0.5	75.8 ± 0.5
Hydroponic Solution	75.0 ± 0.6	75.1 ± 0.5

† Significantly different from other means at the .05 level based on paired T tests.

± The 95% confidence interval.

It was concluded from experimentation in a controlled environment that tritium was fractionated at several sites in plants. Discrimination against tritiated water entering plants was not evident but, under conditions of slow transpiration, a small fractionation could be detected in the leaves. Discrimination against tritium in the formation of organic compounds was about twenty-two percent.

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